JOURNAL OF CHEMICAL EDUCATION

Laboratory Experiment

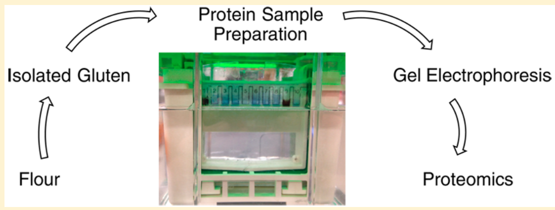
pubs.acs.org/jchemeduc

Introducing Students to Protein Analysis Techniques: Separation and Comparative Analysis of Gluten Proteins in Various Wheat Strains

Alyssa L. Pirinelli, Jonathan C. Trinidad, and Nicola L. B. Pohl*

Department of Chemistry, Indiana University, Bloomington, Indiana 47405, United States

Supporting Information



ABSTRACT: Polyacrylamide gel electrophoresis (PAGE) is commonly taught in undergraduate laboratory classes as a traditional method to analyze proteins. An experiment has been developed to teach these basic protein gel skills in the context of gluten protein isolation from various types of wheat flour. A further goal is to relate this technique to current mass spectrometry-based proteomic analysis techniques. Gluten proteins serve as particularly good experimental systems for the undergraduate classroom given the low cost of their sources and the current widespread public interest in gluten-free diets and Celiac-Sprue disease. The experiment set also can serve as preliminary data for student-written original research proposals and be the basis for a discussion of how scientific topics are presented in public forums. This experiment can also be readily adapted to a range of audiences and facilities.

KEYWORDS: *Upper-Division Undergraduate, Biochemistry, Laboratory Instruction, Communication/Writing, Inquiry-Based/Discovery Learning, Electrophoresis, Mass Spectrometry, Proteins/Peptides, Hands-On Learning/Manipulatives*

BACKGROUND

Undergraduate lab experiments commonly use the same experimental conditions year after year with little change in conditions or results. Inquiry-based learning methods are slowly becoming available to replace these older style exercises.[1] Although instructors in charge of general and organic chemistry laboratory classes now have a broad range of options,[2] examples of such exercises applicable to learning common biochemical techniques easily modified for a range of classrooms that showcase active research areas are rare.[3]

Given the lack of data in the scientific literature and the increasing public discussion of Celiac-Sprue and gluten intolerance, wheat is a good platform for developing discovery-based or inquiry-based experiments for undergraduates.[4] Whereas Celiac-Sprue is a recognized medical problem in which patients cannot properly digest gluten and their intestines gradually lose the ability to absorb other nutrients, gluten intolerance is less clearly defined as an illness.[5] Hybrid wheat is the largest source of dietary gluten in the United States and therefore under increasing scrutiny.[4] Different cultivars of wheat (such as the older einkorn, spelt, and Khorasan wheats) could have differing proportions of gliadin and glutenin, the major components of gluten and those that give the products made from flour their texture.[6] Generally, the higher the levels of gluten in wheat flour, the airier and lighter the bread.[7]

Despite the prevalence of wheat in the U.S. diet, the biochemistry, genome sequences and protein makeup of wheat strains are still relatively unexplored, and therefore the differences between modern hybrid wheat and older wheat strains are not yet known quantitatively.[8] Changing the type and source of gluten in bread has been shown to alleviate symptoms in some patients, though the mechanisms and broad applicability of these observations remain unclear.[9] Greater knowledge of the specific components of modern versus older wheat strains could allow the directed evolution of strains that do not cause adverse reactions or the selection of flour types to allow Celiac-Sprue patients to eat wheat products again.[10]

A set of experiments including SDS-PAGE has been developed that extracts gluten proteins from wheat flour to illustrate current protein purification techniques and molecular weight characterization methods (Figure 1). Students are encouraged to propose hypotheses along the following lines: comparative levels of glutenin and gliadin as well as the method and success of various isolation conditions.

This experiment was originally developed for the protein biochemistry portion of a one-year honors-level introductory biology class that includes students from the Intensive Freshman-Learning Experience (IFLE) program at Indiana University Bloomington.[11] The course is designed as an inquiry-driven class with a very low student to faculty ratio (about five students per faculty member with an average class of 11 students). After two successive years in this class (22 students total), the experiment was slightly modified for the first-semester general biochemistry laboratory class with 48 students total divided into four sections. For more information on how the procedure was modified between the types of classes, please see the Supporting Information.

EXPERIMENTAL OVERVIEW

Modern protein analyses often include a PAGE gel; this concept can be taught in the context of the student flour

DOI: 10.1021/acs.jchemed.5b00311
J. Chem. Educ. XXXX, XXX, XXX−XXX

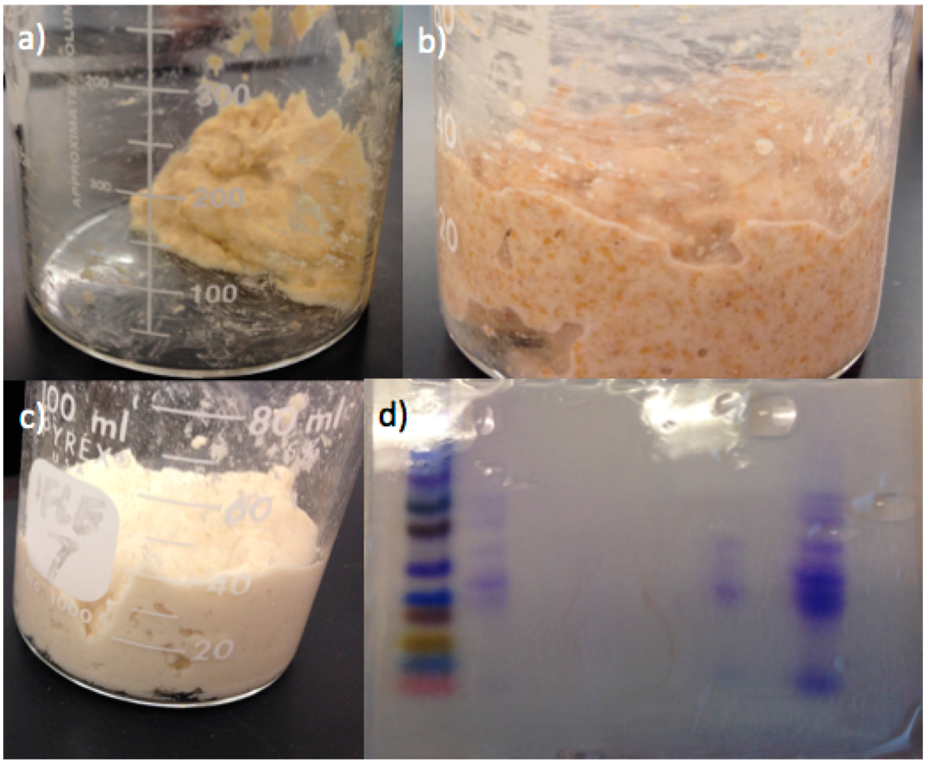


Figure 1. Examples of developing gluten samples: (a) 1:1 (w/w) mixtures of Khorasan wheat (*Triticum turgidum* ssp.) Khorasan, (b) store-brand whole wheat, and (c) store-brand unbleached white flour. (d) An honors biology student gel run on gluten proteins with a molecular weight ladder, at left, as reference. This gel depicts two types of flour, white with salt (left and right), Khorasan flour with no salt (middle), with varying amounts of protein. The protein bands for these lanes show approximately identical proteins.

protein samples and, if budget allows, results can be verified using on- or off-campus mass spectrometry (MS) facilities. State-of-the-art mass spectrometry is capable of getting far more accurate molecular weights than is possible using PAGE. This technique also relies on modern databases of proteins and genome information.[12]

Here, students from either an upper level biochemistry class or an honors freshman class were asked to isolate gluten proteins from flour using a controlled set of varied conditions. Both classes then ran SDS-PAGE gels on their protein samples primarily to learn about the basics of this protein purification method, the entire process being contained within one laboratory session. The honors class was given the opportunity to use in-house mass spectrometry facilities to look at the differences in gluten proteins the following class. To illustrate the requirements of the experiments, an approximate timetable for the entirety of the lab is included in Table 1.

This laboratory experiment is distinct from a normal biochemistry laboratory due to the ability to change the parameters of the experiments. This experiment best fits into two class periods of under 3 h apiece, but can be modified to fit into a single section of around 4 h. If necessary, the work can be paused and the samples refrigerated either after the gluten isolation or after the sample preparation depending on the lab time available to the implementing institution (Table 1). Furthermore, the students are given some license to choose the exact experiment that they run with respect to the gluten isolation conditions, the instructors have the opportunity to present the information to the students in either a formal or informal manner through explanation, and the evaluation methods at the end of the experiment can range from discussions, to quizzes and formal write-ups (examples of discussion and quiz questions, as well as a proposed outline for an NIH-formatted proposal can be found in the Supporting Information).

Table 1. Approximate Lab Completion Times for Experienced and Inexperienced Students, Including Out-of-Class Assignments

		Approximate Class Time, min	
Location	Activity	Upper-Level Undergraduate	Lower-Level Undergraduate
Out of Class	Pre-lab Reading	60	120
Out of Class	Pre-lab Activity	-	60
In Class	Pre-lab Explanation	15	30
In Class	Gluten Isolation	60	60
In Class	Sample Preparation	40	90
In Class	Gel Loading	5	15
In Class	Run Gel	45	45
Out of Class	Post-Lab Activity	90	300

HAZARDS

Excess flour from the experiment and any remaining gluten isolated within a laboratory setting should be labeled as "not for human consumption" and properly stored or disposed of. The dough formation and gluten isolation should be done with gloves to avoid protein contamination.

The instructors should prepare the iodine dye and loading buffer in a fume hood, and, depending on the class dynamic,

DOI: 10.1021/acs.jchemed.5b00311
J. Chem. Educ. XXXX, XXX, XXX−XXX

loading the gels should also be done by the teaching staff. The chemicals (loading dye, buffer systems and stain) for the gel experiment can be used outside of a hood, though the beta-mercaptoethanol should be portioned out in a hood.

RESULTS AND DISCUSSION

Student Performance

Nearly every student isolated substantial protein amounts; the approximate proportion of protein:starch in flour was around 9:1, and most students were able to isolate greater than a gram of protein from 10 g of flour. The exceptions were those who chose saltwater concentrations above 1 M for the dough formation; even these students were able to isolate sufficient protein for use in the gel.

Accounting for the approximate water content in their protein (due to time constraints, the students were not given access to a freeze-dryer to obtain dehydrated protein), the students used a small amount (from 10 to 50 mg) of their isolated gluten protein to make their concentrated gel samples; aliquots from these concentrated solutions were used to create samples with a concentration around 1 mg/mL). Upon dissolution of the isolated protein in the buffer medium, post-run staining showed protein in nearly every lane proving the students' calculations were accurate. No variety of gluten seemed particularly soluble, though the longer time period the students used to dissolve the protein, the more protein appeared on the gel.

Three methods were used to assess students' increased knowledge including two different types of writing assignments and an in-class discussion. After a discussion on gluten, the honors biology students were given quizzes in short answer format to judge the extent of their mastery of the concepts. All of the students gave sufficient answers to show they understood the broader concepts of dilutions, protein isolation, PAGE experiments (sample quiz question examples are given in the Supporting Information). These students were also required to come up with an independent research idea and hypothesis, go into the primary literature for background (here presuming prior database instruction from the on-campus science library staff) and then write a short National Institutes of Health-style proposal. These students were given four additional 3 h lab periods to complete their projects as approved by the course instructors. Though the implementation of the proposals is only possible for a class with sufficient financial and personnel resources, the proposal can serve as a good way to motivate active engagement, allow students practice in scientific persuasive writing, and give students a window into another aspect of the work life of effective scientists.

In this case, the honors biology students all seemed very motivated by the subject matter to propose and perform quality experiments. They asked important questions on their chosen topics, delved into the literature and came up with appropriate methods to test their hypotheses.

The upper-level biochemistry class contained 48 students. They were given a structured, question-based written assignment to determine their understanding of the material. Since the students had been able to work together during the laboratory portion, individual responses with respect to accrued knowledge between the students varied greatly. Although approximately a quarter of the students did not or could not interpret their results correctly with respect to gluten proteins between flours, nearly all (90−95%) of the students understood the basics of running and interpreting PAGE experiments at the completion of the lab. The overall impact of the lab in teaching PAGE was highly successful.

The gel bands showed very little difference between the size and amounts of proteins of the flours tested, which proved many of the students' hypotheses to be false. The classes were asked to explain these results and the advantages of access to a more modern and accurate method of mass determination.

Lessons for Adoption Elsewhere

This lab was designed with intentional flexibility for adoption in a variety of settings. This lab can most easily take the place of the standard "cook-book-style" PAGE experiments found in introductory biochemistry laboratory classes with or without inclusion of the student lab handout or expectation of an original research proposal. However, the experiment could also be easily adapted for other audiences (K−12 or outreach classes) or courses with minimal resources. The differences between the components of flour (starch, protein, etc.) can be highlighted and used to show a wide range of audiences that a staple food such as wheat flour is not necessarily just a simple powder, nor is their isolated "gluten" protein made up of mostly gluten. In these cases, it is possible that the analytical portion of the lab can be foregone for the hands-on portion (gluten isolation) of this lab, and results from an example protein gel or prior proteomics mass spectrometry experiment can be discussed to increase the audience's understanding of the material. Aside from the target audience, many of the other modules within the lab can be modified with respect to available resources, materials and time to accommodate anywhere from 1 to 4 h lab periods (Table 1).

CONCLUSIONS

An SDS-PAGE experiment based on the proteins in wheat flour was designed to help a range of students learn the basics of protein structure and electrophoresis, as well as proteomic mass spectrometry in the context of important "real world" research. This experiment can be easily substituted for a current one-week SDS-PAGE experiment in an introductory biochemistry laboratory class or adapted for a range of situations with different student enrollments, funding and equipment availability. Furthermore, the experiment is readily amenable as a starting point for other activities such as a discussion of the public discourse of science or developing scientific proposal writing skills.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available on the ACS Publications website at DOI: 10.1021/acs.jchemed.5b00311.

Full MS data (XLSX)

Notes to instructors, reagents list, background supporting documents for students, sample questions for assignments, mass spectrometry procedures and sample data, proposal guidelines and honors course quiz questions (PDF)

AUTHOR INFORMATION

Corresponding Author

*E-mail: npohl@indiana.edu.

DOI: 10.1021/acs.jchemed.5b00311
J. Chem. Educ. XXXX, XXX, XXX−XXX

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors would like to thank the Fall 2013 and Fall 2014 H111/H112 classes as well as the Spring 2015 C487 lab associate instructors and students for their participation in this experiment. We would especially like to thank C. E. Dann III for his helpful suggestions on the lab handout. This material is based in part upon work supported by the National Science Foundation under CHE-1362213.

■ REFERENCES

(1) (a) Kossida, S.; Tahri, N.; Daizadeh, I. Bioinformatics by Example: From Sequence to Target. *J. Chem. Educ.* **2002**, *79* (12), 1480−1485. (b) Powers, J. L.; Rippe, K. D.; Imarhia, K.; Swift, A.; Scholten, M.; Islam, N. A Direct, Competitive Enzyme-Linked Immunosorbent Assay (ELISA) as a Quantitative Technique for Small Molecules. *J. Chem. Educ.* **2012**, *89* (12), 1587−1590. (c) Quardokus, R. C.; Wasio, N. A.; Kandel, S. A. The Scanning Theremin Microscope: A Model Scanning Probe Instrument for Hands-On Activities. *J. Chem. Educ.* **2014**, *91* (2), 246−250. (d) Childs-Disney, J. L.; Kauffmann, A. D.; Poplawski, S. G.; Lysiak, D. R.; Stewart, R. J.; Arcadi, J. K.; Dinan, F. J. A Metabolic Murder Mystery: A Case-Based Experiment for the Undergraduate Biochemistry Laboratory. *J. Chem. Educ.* **2010**, *87* (10), 1110−1112. (e) Jensen, M. B. Integrating HPLC and Electrochemistry: A LabVIEW-Based Pulsed Amperometric Detection System. *J. Chem. Educ.* **2002**, *79* (3), 345−348. (f) Bevan, S. J.; Chen, C. W.; Tanner, J. A. Diverse Assessment and Active Student Engagement Sustain Deep Learning: A Comparative Study of Outcomes in Two Parallel Introductory Biochemistry Courses. *Biochem. Mol. Biol. Educ.* **2014**, *42* (6), 474−479. (g) Ott, L. E.; Carson, S. Immunological Tools: Engaging Students in the Use and Analysis of Flow Cytometry and Enzyme-Linked Immunosorbent Assay (ELISA). *Biochem. Mol. Biol. Educ.* **2014**, *42* (5), 382−397. (h) Schwarz, G.; Ickert, S.; Wegner, N.; Nehring, A.; Beck, S.; Tiemann, R.; Linscheid, M. W. Protein Quantification by Elemental Mass Spectrometry: An Experiment for Graduate Students. *J. Chem. Educ.* **2014**, *91* (12), 2167−2170. (i) Timerman, A. P.; Fenrick, A. M.; Zamis, T. M. The Isolation of Invertase from Baker's Yeast: A Four-Part Exercise in Protein Purifcation and Characterization. *J. Chem. Educ.* **2009**, *86* (3), 379−381.

(2) (a) Slade, M. C.; Raker, J. R.; Kobilka, B.; Pohl, N. L. A Research Module for the Organic Chemistry Laboratory: Multistep Synthesis of a Fluorous Dye Molecule. *J. Chem. Educ.* **2014**, *91* (1), 126−130. (b) Hutt, J. T.; Aron, Z. D. Synthesis and Application of Ratiometric and "Turn-On" Fluorescent pH Sensors: An Advanced Organic Undergraduate Laboratory. *J. Chem. Educ.* **2014**, *91* (11), 1990−1994. (c) Reeve, A. M. Reaction of Dimedone and Benzaldehyde: A Discovery-Based Lab for Second-Semester Organic Chemistry. *J. Chem. Educ.* **2015**, *92* (3), 582−585. (d) Hartings, M. R.; Fox, D. M.; Miller, A. E.; Muratore, K. E. A Hybrid Integrated Laboratory and Inquiry-Based Research Experience: Replacing Traditional Laboratory Instruction with a Sustainable Student-Led Research Project. *J. Chem. Educ.* **2015**, *92* (6), 1016−1023.

(3) (a) Furge, L. L.; Boyer, R.; Wolfson, A. J. Innovation in the Biochemistry/Molecular Biology Laboratory. *Biochem. Mol. Biol. Educ.* **2015**, *43* (2), 66−67. (b) Avila, C.; Quesada, A. R. Evaluation of the Applicability of Two Different Immunoassays for the Detection of Wheat Gluten in Baby Foods. *Biochem. Mol. Biol. Educ.* **2000**, *28* (5), 261−264. (c) Taylor, A.; Sajan, S. Testing for Genetically Modified Foods Using PCR. *J. Chem. Educ.* **2005**, *82* (4), 597−598. (d) Thompson, R. Q.; Chu, C.; Gent, R.; Gould, A. P.; Rios, L.; Vertigan, T. M. Visualizing Capsaicinoids: Colorimetric Analysis of Chili Peppers. *J. Chem. Educ.* **2012**, *89* (5), 610−612. (e) Hopkins, T. A.; Samide, M. Using a Thematic Laboratory-Centered Curriculum to Teach General Chemistry. *J. Chem. Educ.* **2013**, *90* (9), 1162−1166.

(4) (a) Weijers, H. A.; van de Kamer, J. H. Coeliac Disease. I. Criticism of the Various Methods of Investigation. *Acta Paediatr.* **1953**, *42* (1), 24−33. (b) Dicke, W. K.; Weijers, H. A.; van de Kamer, J. H. Coeliac Disease. II. The Presence in Wheat of a Factor Having a Deleterious Effect in Cases of Coeliac Disease. *Acta Paediatr.* **1953**, *42* (1), 34. (c) USDA ERS. Wheat: Wheat's Role in the U.S. Diet. http://www.ers.usda.gov/topics/crops/wheat/wheats-role-in-the-us-diet.aspx (accessed Apr 2015). (d) Ludvigsson, J. F.; Bai, J. C.; Biagi, F.; Card, T. R.; Ciacci, C.; Ciclitira, P. J.; Green, P. H.; Hadjivassiliou, M.; Holdoway, A.; van Heel, D. A.; Kaukinen, K.; Leffler, D. A.; Leonard, J. N.; Lundin, K. E.; McGough, N.; Davidson, M.; Murray, J. A.; Swift, G. L.; Walker, M. M.; Zingone, F.; Sanders, D. S. Diagnosis and Management of Adult Coeliac Disease: Guidelines from the British Society of Gastroenterology. *Gut* **2014**, *63* (8), 1210−1228. (e) Schuette, H. A. Know Your Foods. II. Graham Bread. *J. Chem. Educ.* **1929**, *6* (1), 59−64. (f) Ludvigsson, J. F.; Green, P. H. The Missing Environmental Factor in Celiac Disease. *N. Engl. J. Med.* **2014**, *371* (14), 1341−1343.

(5) (a) Czaya-Bulsa, G. Non-Coeliac Gluten Sensitivity − A New Disease with Gluten Intolerance. *Clin. Nutr.* **2015**, *34* (2), 189−194. (b) Nijeboer, P.; Bontkes, H. J.; Mulder, C. J.; Bouma, G. J. Non-Celiac Gluten Sensitivity. Is it in the Gluten or the Grain? *J. Gastroinestin Liver Dis.* **2013**, *22* (4), 435−440. (c) Carroccio, A.; Mansueto, P.; Iacono, G.; Soresi, M.; D'Alcamo, A.; Cavataio, F.; Brusca, I.; Florena, A. M.; Ambrosiano, G.; Seidita, A.; Pirrone, G.; Battista Rini, G. B. Non-Celiac Wheat Sensitivity Diagnosed by Double-Blind Placebo-Controlled Challenge: Exploring a New Clinical Entity. *Am. J. Gastroenterol.* **2012**, *107* (12), 1898−1906. (d) Catassi, C.; Bai, J. C.; Bonaz, B.; Bouma, G.; Calabrò, A.; Carroccio, A.; Castillejo, G.; Ciacci, C.; Cristofor, F.; Dolinsek, J.; Francavilla, R.; Elli, L.; Green, P.; Holtmeier, W.; Koehler, P.; Koletzko, S.; Meinhold, C.; Sander, D.; Schumann, M.; Schuppan, D.; Ullrich, R.; Vécsei, A.; Volta, U.; Zevallos, V.; Sapone, A.; Fasano, A. Non-Celiac Gluten Sensitivity: The New Frontier of Gluten Related Disorders. *Nutrients* **2013**, *5* (10), 3839−3853. (e) Vanga, R.; Leffler, D. A. Gluten Sensitivity: Not Celiac and Not Certain. *Gastroenterology* **2013**, *145* (2), 276−279. (f) Tonutti, E.; Bizzaro, N. Diagnosis and Classification of Celiac Disease and Gluten Sensitivity. *Autoimmun. Rev.* **2014**, *13* (4−5), 472−476.

(6) Sliwinski, E. L.; Kolster, P.; Prins, A.; van Vliet, T. On the Relationship Between Gluten Protein Composition of Wheat Flours and Large-Deformation Properties of Their Doughs. *J. Cereal Sci.* **2004**, *39* (2), 247−264.

(7) Pollan, M. *Cooked: A Natural History of Transformation*, 1st ed.; The Penguin Press: New York, NY, 2013; pp 203−290.

(8) (a) Eversole, K.; Feuillet, C.; Mayer, K. F.; Rogers, J. Slicing the Wheat Genome. *Science* **2014**, *345* (6194), 285−287. (b) For more information on the wheat genome, please go to www.wheatgenome.org.

(9) (a) Manu, B. T.; Prasada Rao, U. J. Role of Peroxidase and H_2O_2 in Cross-Linking of Gluten Proteins. *J. Food Biochem.* **2011**, *35*, 1695−1702. (b) Tanabe, S.; Watanabe, M.; Arai, S. Production of a High-Glutamine Oligopeptide Fraction from Gluten by Enzymatic Treatment and Evaluation of its Nutritional Effect on the Small Intestine of Rats. *J. Food Biochem.* **1992**, *16* (4), 235−248. (c) Wang, J.- S.; Zhao, M.- M.; Bao, Y.; Hong, T.; Rosella, C. M. Preparation and Characterization of Modified Wheat Gluten by Enzymatic Hydrolysis-Ultrafiltration. *J. Food Biochem.* **2008**, *32* (3), 316−334. (d) van den Broeck, H. C.; van Herpen, T. W.; Schuit, C.; Salentijn, E. M.; Dekking, L.; Bosch, D.; Hamer, R. J.; Smulders, M. J.; Gilissen, L. J.; van der Meer, I. M. Removing Celiac Disease-Related Gluten Proteins from Bread Wheat While Retaining Technological Properties: A Study with Chinese Spring Deletion Lines. *BMC Plant Biol.* **2009**, *9*, 41.

(10) (a) van den Broeck, H.; Hongbing, C.; Lacaze, X.; Dusautoir, J.-C.; Gilissen, L.; Smulders, M.; van der Meer, I. In Search of Tetraploid Wheat Accessions Reduced in Celiac Disease-Related Gluten Epitopes. *Mol. BioSyst.* **2010**, *6* (11), 2206−2213. (b) Salentijn, E. M.;

DOI: 10.1021/acs.jchemed.5b00311
J. Chem. Educ. XXXX, XXX, XXX−XXX

Goryunova, S. V.; Bas, N.; van der Meer, I. M.; van den Broeck, H. C.; Bastien, T.; Gilissen, L. J.; Smulders, M. J. Tetraploid and Hexaploid Wheat Varieties Reveal Large Differences in Expression of Alpha-Gliadins from Homologous Gli-2 loci. *BMC Genomics* **2009**, *10*, 48.

(11) College of Arts and Sciences: Office of Science Outreach STRS & IFLE Undergraduate Research Programs. http://college.indiana.edu/science/undergradscienceresearch/ifle.shtml (accessed Nov 2015).

(12) (a) Yates, J. R.; Ruse, C. I.; Nakorchevsky, A. Proteomics by Mass Spectrometry: Approaches, Advances, and Applications. *Annu. Rev. Biomed. Eng.* **2009**, *11*, 49−79. (b) Otto, A.; Becher, D.; Schmidt, F. Quantitative Proteomics in the Field of Microbiology. *Proteomics* **2014**, *14* (4−5), 547−565. (c) Kim, D. H.; Eckhert, C. D.; Faull, K. F. Utilization of Negative Ion ESI-MS and Tandem Mass Spectrometry to Detect and Confirm the NADH-Boric Acid Complex. *J. Chem. Educ.* **2011**, *88* (1), 106−110. (d) Gross, D. S.; Van Ryswyk, H. Examination and Manipulation of Protein Surface Charge in Solution with Electrospray Ionization Mass Spectrometry. *J. Chem. Educ.* **2014**, *91* (8), 1240−1243.

DOI: 10.1021/acs.jchemed.5b00311
J. Chem. Educ. XXXX, XXX, XXX−XXX